



In research that may impact the treatment of several diseases, scientists are using molecules that can bind to and activate (or inhibit) human proteins called adenosine receptors to study receptor signaling. Shown is a computer-generated model illustrating how one such activating molecule (an “agonist”) binds to one subtype of adenosine receptor. The agonist is represented as a very small ball-and-stick model within its binding site on the receptor. For more information on this exciting research, see the Feature on “Insights into Cellular Communication Through Medicinal Chemistry.” Image courtesy of Dr. Ken Jacobson, Chief, Molecular Recognition Section, NIDDK Division of Intramural Research.

Cross-Cutting Science

The tale of scientific discovery is told in incremental steps, with each new insight adding another page to the story. Often, this research focuses on the fundamental, microscopic and molecular components of an organism—its DNA, genes, proteins, and metabolites—and the exquisitely complex ways in which these elements are organized, regulated, and interact. While its ultimate application may not always be immediately obvious, this research can form a crucial foundation for future investigations, and insights gained from this research can be expected to facilitate disease-based research in a wide range of fields. A critically important aspect of cross-cutting research is the effort to translate research advances made in the laboratory into more effective therapies for patients, and to use insights gained from clinical studies to spur novel research directions in the laboratory.

STRENGTHENING RESEARCH ACROSS THE NIDDK

From Bench to Bedside – New Efforts to Accelerate Translational Research: Shepherding research from the laboratory—the “bench” at which a scientist performs experiments—to the doctor’s office or hospital room—the “bedside” at which the patient is treated—is an important component of trans-NIDDK research. “Bench to bedside” is one aspect of “translational research,” the movement of knowledge gained from laboratory research studies into the realm of clinical studies. Translation can also refer to efforts to bring insights gained from clinical trials to changes in the practice of medicine on a large scale to effect improvements in public health. Ultimately, investigators seek to move research findings from clinical study to healthcare practice in the community or public-health arena, a progression termed “bedside to practice.”

The reverse process, “bedside to bench research,” (or bench to bedside and back) is also valuable, as it brings knowledge gained in a clinical setting back to the laboratory for further exploration that may in turn spur new clinical endeavors. Ideally, knowledge flows in both directions, with research insights translating into improvements in patient care, and with clinical studies and observation catalyzing new lines of investigation at the laboratory bench. Translational research is an overarching theme of

this document, highlighted especially in the Stories of Discovery and other advances in this document, and represented on the cover.

The NIDDK has undertaken new efforts to bolster translational research. In early FY 2004, the NIDDK Director established the Translational Research Working Group to identify obstacles to translational research and to develop ways of overcoming them, to identify opportunities, and to assess translational research priorities. Although the Working Group is focused on bench-to-bedside research, the NIDDK also vigorously supports translational research from bedside to practice. Such efforts include, for example, ongoing research demonstration and dissemination projects to explore strategies to effectively bring results of major clinical trials to patient care and the public, and an FY 2004 conference to discuss the science of translating diabetes and obesity research from clinical trials to the community and future research directions.

In developing new translational research efforts, the Translational Research Working Group sought external input from the NIDDK’s National Advisory Council through discussions at its 2004 meetings, and other sources. Key areas for intensified research that emerged include:

- Enhancing the development of biomarkers (for example, particular biological molecules or patterns

that would reflect disease progression prior to the appearance of actual disease symptoms and that could thus improve monitoring of the effects of experimental treatment strategies);

- Developing new imaging methods;
- Generating new animal models for preclinical research on NIDDK-relevant diseases;
- Research on angiogenesis (blood vessel growth) for diabetic complications and islet transplantation;
- Research on an important effect of elevated blood sugar levels—the overproduction of reactive oxygen species in cellular components called mitochondria;
- Developing therapeutic agents for diseases characterized by protein misprocessing and misfolding; and
- Further research on a type of molecule called RNAi, which may have therapeutic potential.

Many of these areas would have implications for diseases across the mission of the NIDDK.

New Cross-cutting Efforts: The NIDDK is pursuing new broad-based opportunities to advance research on many diseases. For example, an initiative launched this past year is encouraging research to understand and mitigate issues of health disparities in diseases within the mission of the NIDDK. Another new initiative will support projects that advance the use of “proteomic” approaches in research on diabetes, obesity, and endocrine, digestive, kidney, urologic, and hematologic diseases. Proteomic technologies focus on proteins, just as genomic approaches focus on genes (and specifically on the DNA of which genes are made, and on the RNA intermediates between genes and the proteins they ultimately encode). Proteomics will help shed new light on biological processes in health and disease by enhancing understanding of patterns of protein production, interactions among proteins, and other aspects of proteins in cells, tissues, and organ systems. Also relevant to diseases across its mission, the Institute began an initiative in 2004 to encourage the development of assays for high throughput drug screening.

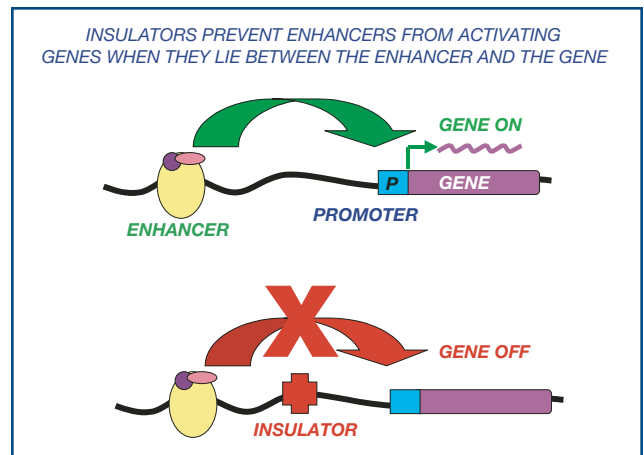
As part of the NIDDK’s Division of Intramural Research, three laboratories—devoted to chemical synthesis, biological screening, and computational chemistry—are working together on a “Chemical Biology” initiative to develop small molecule reagents. These reagents would first be used in NIDDK scientists’ research, and the reagents could eventually be optimized further for potential use as drugs. This initiative fosters interdisciplinary research approaches to important biological problems.

Research Training – Ensuring a Pipeline of

Investigators for the Future: A critical component of NIDDK’s research efforts is investigator training. Among the Institute’s research training efforts are those focused on minority investigators, to advance scientific knowledge and to help reduce racial and ethnic health disparities. The Institute’s Office of Minority Health Research Coordination established the Network of Minority Research Investigators (NMRI). The major objective of the NMRI is to encourage and facilitate participation of members of underrepresented racial and ethnic minority groups in the conduct of biomedical research in fields relevant to the NIDDK’s mission. The NMRI is a communication network of current and potential biomedical research investigators and technical personnel from traditionally underserved communities: African American, Hispanic American, American Indian, Alaska Native, Native Hawaiian, and other Pacific Islanders. The major objective of the network is to encourage and facilitate participation of members of underrepresented racial and ethnic minority groups in the conduct of biomedical research in the fields of diabetes, endocrinology, metabolism, digestive diseases, nutrition, kidney, urologic, and hematologic diseases. A second objective is to encourage and enhance the potential of the underrepresented minority investigators in choosing a biomedical research career in these fields. An important component of this Network is promotion of two-way communications between network members and the NIDDK. The primary goals of the Network are to help minority investigators achieve career success while working on issues concerning health-related racial and ethnic disparities.

The NIDDK also supports educational efforts at the college and pre-college levels. For example, the Diabetes-based Science Education in Tribal Schools (DETS) Program is intended to promote a diabetes-based science curriculum that will enhance understanding and appreciation of the problems of diabetes in American Indian communities, and will stimulate general student interest in diabetes-based science in the early years (pre-college) of education. Selected tribal colleges and universities have been funded to develop supplemental curricula for K-12 schools in American Indian and Alaska Native communities. The investigators have embarked on developing three parallel curricula, K-4, 5-8 and 9-12, which will be sequential and interrelated to give a continuum of exposure to diabetes-based science education. The NIDDK, the Centers for Disease Control and Prevention (CDC), and the Indian Health Service (IHS) are jointly supporting the program. Through better understanding of diabetes, tribal children can be instrumental in preventing the development of and in better managing diabetes, and reducing its human costs. This goal can be better achieved through the entry of greater numbers of tribal children into the health-science professions.

Among other training programs is the NIDDK's Summer Internship Program (SIP), which provides an opportunity for undergraduate students to participate in research under the direction of preceptors in NIDDK laboratories. The purpose of this program is to advance the state of biomedical knowledge, and to introduce the students to current laboratory methods in the field. The National High School Student Summer Research Program is designed to enhance exposure to basic and clinical research, and academic medicine, as viable and desirable career choices, among a pool of underrepresented minority high school students. Such a program serves as an early component of the pipeline that brings students from economically disadvantaged backgrounds and medically under-served populations to the corridors of academia, which, in turn, focus on producing scientists, physicians, and allied health professionals to practice in their respective communities.



“Insulator” elements are naturally-occurring segments of DNA that mark boundaries in certain regions of the genome so as to enable a cell to regulate neighboring genes independently. Insulator elements have two functions, the first of which is referred to as enhancer-blocking activity, as shown. An enhancer is a sequence of DNA that directs the cell to turn on an associated gene. If positioned near an enhancer, an insulator can prevent the enhancer's signal from being broadcast in the wrong direction and thus keep the cell from turning on genes that it shouldn't. The second function of insulators is to set up a “barrier” to prevent unwanted silencing—or tight shutting off—of genes. Image courtesy of Dr. Gary Felsenfeld, NIDDK Division of Intramural Research. For more information, see page 9.

NIH Roadmap for Medical Research in the 21st Century: The NIH Roadmap provides a framework of the priorities the NIH as a whole must address in order to optimize its entire research portfolio. It identifies the most compelling opportunities in three main areas: New Pathways to Discovery, Research Teams of the Future, and Re-engineering the Clinical Research Enterprise. The following are highlights of a few of the many Roadmap initiatives.

The NIDDK has a leadership role for an NIH Roadmap initiative on “Metabolomics Technology Development.” The “metabolome” is the complete set of small molecules in the body which function as nutrients, chemical signals and building blocks such as amino acids, peptides, and lipids. “Metabolomics” is the study of these small molecules. The purpose of this initiative is to promote the development of highly innovative and sensitive tools for studying metabolomics. The development of such novel

technologies can directly benefit the study of diseases within the NIDDK mission. For example, metabolomics could lead to the identification and validation of surrogate markers that correlate with stage or rate of progression of diabetes and its complications. Furthermore, metabolomics technologies could be applied to the development of novel, less-burdensome diagnostic tests for pre-diabetes and type 2 diabetes.

Another Roadmap initiative on “Interdisciplinary Research” aims to overcome the current barriers that prevent experts from different fields from working together to advance medical research. Obesity—which is a serious risk factor for type 2 diabetes—is a key example of a disease that could benefit from increased partnerships among different communities. The increase in obesity has been fueled by a complex interplay of environmental, social, economic, and behavioral factors, acting on a background of genetic susceptibility. Therefore, researchers with expertise in numerous disciplines—such as genetics, behavioral science, and biochemistry—can offer important contributions to obesity research. Another example of an initiative that will benefit NIDDK programs is the establishment of “translational research core services” to promote translation of novel therapeutics from the bench-to-the-bedside by providing access to sophisticated manufacturing capacity and expert advice to ensure that drug-development regulations are observed.

BASIC RESEARCH – CLINICAL IMPLICATIONS

2004 Nobel Prize in Chemistry Awarded for the Discovery of Ubiquitin-Mediated Protein Degradation:

The 2004 Nobel Prize in Chemistry was awarded to three scientists for their discovery of ubiquitin-mediated protein degradation, a process that regulates protein destruction inside a cell. The winners are Dr. Aaron Ciechanover and Dr. Avram Hershko of Technion-Israel Institute of Technology in Haifa, Israel; and Dr. Irwin Rose of the University of California, Irvine. Drs. Rose and Hershko have previously received funding from the NIDDK.

In the early 1970s, Dr. Irwin Rose, who was then at the Fox Chase Cancer Center in Philadelphia, and Dr. Avram Hershko of Technion, were independently studying various research areas related to protein activity in the cell and the relationship to various syndromes, disorders, and diseases. When the two men met at a scientific conference at the NIH in 1976, they realized that they were both interested in the same research question: How does the cell select certain proteins for disassembly into their component parts, contributing to the regulation of protein activity within the cell? Much of the research in the field of cellular biochemistry up to that point had focused on the production of proteins in the cell, not the destruction of them. What interested both men was why it took energy (in the form of adenosine triphosphate, ATP, the energy currency of the cell) to break down these proteins, when in every other arena of biochemistry (such as digestion) energy is not needed for protein degradation. This enigma was too irresistible for the two scientists to ignore.

Out of this interest grew a research collaboration between Drs. Hershko and Rose, and later Aaron Ciechanover, one of Hershko’s graduate students at Technion. The trio worked on the problem separately and together during several summer sabbaticals that Drs. Hershko and Ciechanover spent as visiting scientists in Dr. Rose’s laboratory at Fox Chase Cancer Center.

What they discovered was that protein degradation in the cell is a highly selective activity regulated through a multi-step process that involves “tagging” a protein slated for destruction with a marker. Once a protein undergoes this process, the tagged protein is then chaperoned to the proteasome, the garbage disposal of the cell, for disassembly into its amino acid parts, which can then be reused to make other proteins. Drs. Ciechanover, Hershko, and Rose discovered that the marker that tagged proteins for destruction was the protein ubiquitin, a small polypeptide of only 76 amino acids.

Ubiquitin was first isolated in 1975 from bovine thymus in an NIH-funded study and reported by Gideon Goldstein, *et al.*, in an article in which the authors referred to the newly discovered protein as “ubiquitous immunopoietic polypeptide (UBIP) because it is widespread and perhaps even universally represented in living cells” (*Proc Natl Acad Sci USA* 72: 11-15, 1975). It soon became apparent that this protein was a staple of eukaryotic cells (cells with a defined nucleus) and found in most higher organisms, including plants, fungi, yeast, and animals.

The researchers had hypothesized that the energy requirement for protein destruction was needed for the cell to maintain specificity and control over the process of protein destruction. The question was how the cell used the energy to do this. Drs. Ciechanover, Hershko, and Rose answered this question through a series of pioneering biochemical studies throughout the late 1970s and early 1980s that led to the elucidation of the ubiquitin-mediated protein degradation system. They made their seminal discoveries in 1979 and reported their findings in two papers published in 1980 in the *Proceeding of the National Academies of Science of the United States of America*.^{1,2}

They discovered that the tagging of a protein for destruction was a multi-step process that begins with the creation of a stable, high-energy covalent bond between ubiquitin and an enzyme, labeled E1. The creation of this covalent bond, the strongest kind of chemical bond, requires the input of significant energy in the form of ATP. This was the energy requirement that had so puzzled Drs. Hershko and Rose at the outset of their research. The discovery of the role of covalent bonding surprised the researchers, because most protein-protein interactions that take place inside a cell involve temporary bonding between molecules through weak chemical attractions, such as hydrogen bonding.

The researchers showed that the next step in the process involves the transfer of the ubiquitin protein from the first enzyme, E1, to a second enzyme, E2. The E2-ubiquitin complex then binds to another complex—a third enzyme, E3, and its corresponding target protein, the protein slated for destruction. An important discovery was that it is the specificity of the E3 enzyme that determines which proteins are degraded. Although the E1 and E2 enzymes are relatively similar through all cell types and organisms, there are hundreds of different E3 enzymes within each cell, each one programmed to recognize and bind to a specific protein.

For a brief time, all four components are bound together in very close proximity. Then the ubiquitin binds to the target protein, releasing the E2 enzyme, followed by the release of the E3 enzyme. Through this process, the target protein is tagged with the ubiquitin molecule. Another surprise came when the researchers discovered that the process didn’t stop there. The binding of one ubiquitin molecule to the target protein apparently was not enough. They discovered that the last step of the process is repeated many times until a long chain of ubiquitin molecules is attached to the target protein. They termed this part of the process “polyubiquitination.” This ubiquitin chain chaperones the target protein to the proteasome where it acts as the passkey for entry. The proteasome assists with the entry of the target protein into the barrel-shaped organelle, meanwhile releasing the ubiquitin to go about its business. Once inside the proteasome, the target protein is degraded into short peptide chains of seven to nine amino acids.

Drs. Ciechanover, Hershko, and Rose had worked out most of the details of the multi-step ubiquitin-tagging process by 1983. However, other work later brought to light insights into the ways that ubiquitin-mediated protein degradation contributes to the

general homeostasis of the cell, as well as other roles of the ubiquitin protein in the cell. For example, not only does the cell use the ubiquitin system to modulate protein concentrations according to the need for protein activity, but recent research has shown the system's other roles. Up to 30 percent of newly-produced proteins are immediately destroyed using the ubiquitin system, possibly because they are poorly formed or malfunctioning. Ubiquitin is also an important player in the regulation of the cell cycle, DNA repair, maintenance of chromosome structure, programmed cell death (apoptosis), and immune and inflammatory reactions. Current and future research will focus on developing drugs to intercede at various points in the pathway, either to destroy unwanted proteins or to prevent the destruction of critically needed proteins.

The NIDDK supports a broad program of research on intracellular protein-protein interactions, including study of the role of ubiquitin in protein degradation and other cellular functions. Dr. Avram Hershko received funding from NIDDK, from 1980 to 1995, for the study of the mechanisms of intracellular protein breakdown. Dr. Irwin Rose received funding from the National Institute of Arthritis, Diabetes, Digestive, and Kidney Diseases, the predecessor of the NIDDK, from 1973 to 1982, for the study of carbohydrate metabolism and the control of hexokinase loss in diabetes.

¹ Ciechanover A, Heller H, Elias S, Haas AL, and A Hershko. ATP-dependent conjugation of reticulocyte proteins with the polypeptide required for protein degradation. *Proc Natl Acad Sci USA* 77: 1365-1368, 1980.

² Hershko A, Ciechanover A, Heller H, Haas AL, and IA Rose. Proposed role of ATP in protein breakdown: Conjugation of proteins with multiple chains of the polypeptide of ATP-dependent proteolysis. *Proc Natl Acad Sci USA* 77: 1783-1786, 1980.

2004 Albert Lasker Award for Basic Medical Research for Work on Nuclear Hormone Receptors:

The 2004 Albert Lasker Award for Basic Medical Research was awarded to three scientists for the discovery of a superfamily of nuclear hormone receptors and elucidation of a unifying mechanism that regulates embryonic development and diverse medical pathways. The winners are Dr. Pierre Chambon of the Institute of Genetics and Molecular and Cellular Biology in Strasbourg, France; Dr. Ronald M. Evans of the Salk Institute for Biological Studies in San Diego, California; and Dr. Elwood V. Jensen of the University of Chicago and University of Cincinnati College of Medicine. Their award-winning research, funded in part by the NIDDK, was in the field of nuclear hormone receptors, an area of research of special interest to the Institute.

The winners received the award for the identification of a common cellular mechanism through which a diverse group of hormones, i.e., chemical signaling molecules, regulates a wide range of physiological responses throughout the life-span of an organism. Their research on rodents and humans showed how hormones regulate gene activity through the activation of the hormones' corresponding hormone receptors in the nucleus of a cell. The researchers discovered that there is a "superfamily" of hormones that have corresponding receptors in the nucleus of the cell. This superfamily includes steroid hormones, thyroid hormones, and fat-soluble molecules such as Vitamins A and D. There are also many known nuclear hormone receptors with unidentified corresponding hormone activators—the so-called "orphan nuclear receptors."

Dr. Jensen, who received significant funding from the NIH's National Cancer Institute (NCI), set the stage for this discovery, in the 1950s, with his work on estrogen, a steroid hormone. He discovered that estrogen can activate certain genes inside the nucleus of a cell by binding to its nuclear receptor. Later work by Dr. Jensen and others identified the nuclear receptors

for other steroid hormones, such as testosterone, progesterone, glucocorticoids, aldosterone, and Vitamin D, which has steroid properties. His research ultimately led him to investigate the role of estrogen receptors in breast cancer tumors and led to the use of tamoxifen, an anti-estrogen compound, for the treatment of breast cancer.

By the 1980s, Drs. Chambon and Evans had expanded on Dr. Jensen's work by examining how molecular endocrinology influences gene control—i.e., how hormones turn genes on and off. By early 1986, working independently, Drs. Chambon and Evans discovered the genes for two important nuclear hormone receptors, the estrogen and the glucocorticoid nuclear receptors, respectively. In the same year, they independently discovered a nuclear hormone receptor for retinoic acid, also known as Vitamin A. They later discovered that the receptor, which Dr. Evans named Retinoid X Receptor (RXR), had unique physiological properties and could be used to identify the corresponding hormone for many orphan nuclear receptors. Perhaps Dr. Evans' and Dr. Chambon's greatest contribution to the field of research was in the refining of this technique using RXR to identify orphan nuclear receptors' corresponding hormones. Many of the orphan nuclear receptor complexes that have been "adopted" through this technique have applications to research of specific chronic diseases, including type 2 diabetes and lipid-related disorders.

Many NIH components contributed funding to the research of Drs. Jensen, Evans, and Chambon, including the NIDDK, the NCI, the National Institute on Aging (NIA), the National Institute of Child Health and Human Development, the National Institute of General Medical Sciences, the National Heart, Lung, and Blood Institute, and the National Center for Research Resources. Dr. Ronald M. Evans is a Howard Hughes Medical Institute Investigator.

Nuclear Receptor Signaling Atlas (NURSA), A Trans-NIH Initiative

Currently, the NIDDK is leading an initiative to encourage research on nuclear hormone receptors and to apply discoveries in this field to other fields, including disease-targeted research. The Nuclear Receptor Signaling Atlas (NURSA) is a trans-NIH initiative designed to develop a comprehensive understanding of the structure, function, and role in disease of nuclear hormone receptors, with particular focus on metabolism and the development of a number of metabolic disorders, including type 2 diabetes, obesity, lipid dysregulation, and others, as well as in processes of aging and hormone-dependent cancers.

Dr. Evans leads NURSA as co-Director with colleague, Dr. Bert W. O'Malley of Baylor College of Medicine in Houston, Texas. The initial focus of NURSA was in the area of orphan nuclear receptors, but it soon expanded to include research on all nuclear receptors. The hope is that research into the role of nuclear receptors on genetic expression, and elucidation of the mechanisms of action through which this occurs, will lead to better understanding of some of the diseases that are at the core of the NIDDK and NIH mission—including obesity, diabetes and its complications, osteoporosis, hormone dependent cancers, and digestive diseases.

NURSA began as a request for applications (RFA) that was issued by the NIDDK in June 2001—"A Functional Atlas of Orphan Nuclear Receptors." The RFA was funded in August 2002 as a consortium agreement between the NIH and five institutions: Baylor College of Medicine, Salk Institute, Duke University, the University of Pennsylvania, and the University of Texas Southwestern. The NURSA consortium exists as a cooperative agreement comprising research projects and core resources, funded by three NIH institutes: the NIDDK, the

NCI, and the NIA. Today, NURSA comprises the three original NIH institutes and the five original academic institutions, as well as three other academic institutions: Van Andel Institute, the Beckman Research Institute, and the University of Rochester.

A Straightforward Chemical Modification with Profound Implications: What do toothpaste, salad dressing, and laundry detergent have in common with cutting-edge treatments for diseases such as Crohn's disease and hepatitis C? A lengthy molecule, polyethylene glycol. Used in food and household products as a thickener, polyethylene glycol—also known as PEG—is used by researchers and clinicians to improve the durability of drugs to treat a number of debilitating conditions, including several within the NIDDK research mission.

Chemically, PEG is a long chain of molecules containing carbon, hydrogen, and oxygen atoms. Because of the electric charges on these atoms, PEG attracts an extensive retinue of water molecules when dissolved in an aqueous solution, and the lengthy hydrocarbon chain is effectively “coated” with them. In the late 1970s, researchers found that attaching molecules of PEG to biomolecules—a process known as “pegylation”—increased the molecules' ability to dissolve in water and protected them from enzymatic degradation. In the body, pegylated molecules are protected from immune response and other clearance mechanisms, which has the effect of lengthening the time the drug persists in the body. This effect in turn means that people require fewer doses of a drug and that drugs can accumulate to higher therapeutic levels in the body than would be possible otherwise.

In 1987, NIDDK-supported scientists studying the rare, inherited disease adenosine deaminase (ADA) deficiency reported that weekly injections of pegylated adenosine deaminase could be an effective short-term treatment. Children with ADA deficiency are unable to properly metabolize the nucleotide adenosine because they lack a critical enzyme, and as a consequence, their immune

systems break down. In extreme cases, a condition known as severe combined immunodeficiency can develop, and these patients may have to live in a protected environment to avoid exposure to infectious agents in the environment. At the time of the trial, the primary therapy for ADA deficiency was regular blood transfusions to provide the missing enzyme. However, these transfusions were not always effective, and they carried risks of iron overload or viral infection. The finding that ADA deficiency could be treated with once-a-week injections of pegylated adenosine deaminase was not only an important advance in the treatment of this serious disease, but also a demonstration of the viability of pegylation as a therapeutic strategy.

In the intervening years, pegylation has been used to prolong the beneficial activity of a wide range of drugs and has had a significant impact on the treatment of a number of diseases. For hepatitis C, a pegylated form of the protein interferon is now part of the standard therapy, along with an antiviral drug. Although many patients respond to this therapy, others (especially African Americans) have low response rates, and the virus continues to cause liver damage. The NIDDK is therefore sponsoring a clinical trial, HALT-C, to determine if long-term treatment with pegylated interferon therapy is beneficial to patients who have not responded to initial therapy. Additionally, industry is sponsoring a number of trials of pegylated agents, including a pegylated tumor necrosis factor-alpha antibody for the treatment of Crohn's disease.

Over the past 20 years, pegylation has evolved from a tool in the research laboratory to an effective approach to treatment augmentation. Pegylated drugs are generally safe and effective, and pegylation has emerged as the favored way to improve the staying power, and hence the effectiveness, of a variety of compounds. As for the future, studies are under way to examine the possible benefits of pegylating molecules such as insulin, to prolong its circulation time; antibodies for targeting of tumors; and other enzymes to aid in recovery from injury.

Marking Boundaries on the Blueprint of Life:

At any given time, in any given type of cell, some genes are kept off, while others are turned on, that is, actively read by the cell to generate the products they encode. A group of NIDDK's intramural scientists has been elucidating one way by which this differential regulation is achieved: the activity of "insulator" elements. Insulator elements are segments of DNA that mark boundaries in certain regions of the genome so that neighboring genes can be regulated independently. Originally discovered in fruit flies, insulators also exist in animals and humans. In their investigations, the scientists particularly focused on a pair of insulators that flank a set of beta-globin genes (beta-globin is used in blood cells).

Insulator elements have two functions, the first of which is referred to as enhancer-blocking activity. An enhancer is a sequence of DNA that directs the cell to turn on an associated gene. If positioned near an enhancer, an insulator can prevent the enhancer's signal from being broadcast in the wrong direction and thus keep the cell from turning on genes that it shouldn't. Through studies of an insulator near the beta-globin locus, the scientists discovered a core segment responsible for the enhancer-blocking activity. By attracting a protein called CTCF and other factors that work with it, this part of the insulator keeps an enhancer focused on the correct gene. In other experiments, the scientists had discovered that insulators and CTCF also play an important role in a regulatory phenomenon called "imprinting," which controls certain genes. An imprinted gene is turned on or off depending upon whether the chromosome on which it's located was inherited from the mother or father. The scientists examined a different chromosomal region (not the beta-globin locus) containing an imprinted gene with an insulator element between it and a nearby enhancer. Through a complex process, cells modify the insulator only on the paternally-inherited copy of this chromosome. This modification restricts access of CTCF to the insulator, and thus prevents it from blocking the enhancer.

The second function of insulators is to set up a "barrier" to prevent unwanted silencing—or tight shutting off—of genes. All of a cell's DNA, including the segment with beta-globin genes, is packaged to some extent with various proteins, called histones, into a structure called "chromatin." Chromatin serves not only to keep the cell's long strands of DNA from becoming tangled and unwieldy, but also as a dynamic structure that helps regulate whether and when genes are turned on or off. One way a cell can keep certain genes and other unneeded segments of DNA turned off is to pack them into a highly condensed form of chromatin called "heterochromatin." A potential danger to this action, however, is that the packaging mechanism might reach out too far along a stretch of DNA, grab genes that should be turned on, and bury them in heterochromatin. Just beyond the beta-globin locus, in fact, is a segment of heterochromatin. An insulator element positioned between these regions of DNA acts as a barrier to keep the heterochromatin from propagating too far, and thus protects the beta-globin locus from being silenced. Recently, the scientists found that part of this insulator recruits certain proteins that chemically modify histones in a way that may halt the formation of heterochromatin. This part of the insulator is distinct from the portion that works to block nearby enhancers. Continued study of insulators and chromatin structure will lead to further insights into the regulation of genes, a process critical for health and development.

Felsenfeld G, Burgess-Beusse B, Farrell C, Gaszner M, Ghirlando R, Huang S, Jin C, Litt M, Magdinier F, Mutskov V, Nakatani Y, Tagami H, West A, and Yusufzai T. Chromatin Boundaries and Chromatin Domains. Cold Spring Harbor Symposia on Quantitative Biology, Symposium 69, Pages 1-6, 2004.

West AG, Huang S, Gaszner M, Litt MD, and Felsenfeld G. Recruitment of histone modifications by USF proteins at a vertebrate barrier element. *Mol Cell* 16: 453-463, 2004.

Insights into Cellular Communication Through Medicinal Chemistry

Although a cup of coffee first thing in the morning is used as an eye-opener by millions, not many people know precisely why or how the caffeine in coffee wakes them up. In fact, a closer look at the molecular actions of caffeine offers a tantalizing peek into a world of complex, interconnected molecular signals. Understanding such signaling processes may hold the key to new treatments for diseases as diverse as asthma, cystic fibrosis, stroke, cardiovascular disease, and glaucoma. Caffeine acts on adenosine receptors, and the molecule that naturally occupies this receptor—and the pathways it activates—are the subject of an ambitious research program in the NIDDK intramural Molecular Recognition Section. This Section, headed by Dr. Ken Jacobson, works at the cutting edge of synthetic organic chemistry and molecular modeling to perform studies of the fundamental nature of molecular signaling through receptors for adenosine. Through studies of “medicinal chemistry,” these scientists are making discoveries that may allow physicians in the future to target treatments for a variety of diseases that will be far more effective and have fewer side effects than ever before.

Connection between Caffeine, Adenosine, and Metabolism

Nearly twenty years ago, researchers identified the biochemical mechanism that explains just how that cup of coffee first thing in the morning works. They identified the molecular receptor on the surface of cells to which caffeine binds. When this naturally-occurring stimulant found in coffee, tea, and chocolate binds to this receptor, it prevents the molecule that naturally occupies this site, adenosine, from binding. Adenosine acts a natural depressant; by displacing it, caffeine removes a “brake.”

With adenosine out of the way, cellular activity increases, nerve cells in the brain begin to fire, blood vessels dilate, the heart beats faster, blood pressure rises, and the liver releases sugar into the bloodstream, producing the “buzz” familiar to millions of coffee drinkers. As the caffeine is slowly metabolized, adenosine re-occupies its receptors, and these physiologic changes slowly reverse. This cycle of metabolic highs and lows helps explain why caffeine is the most widely-consumed mood-altering drug in the world.

Adenosine is a relatively simple, nitrogen- and sugar-containing molecule found throughout the body. It is one of the four components that comprise DNA. It is also the core of adenosine triphosphate, or ATP, a molecular form of energy currency that the cells of the body use to store and release energy. Various forms of adenosine therefore play important roles in information storage (in DNA) and cellular energetics (as ATP and its metabolites). Unmodified adenosine itself also plays an important role in intra- and intercellular signaling and metabolism as well.

Biology of Adenosine and Its Receptors

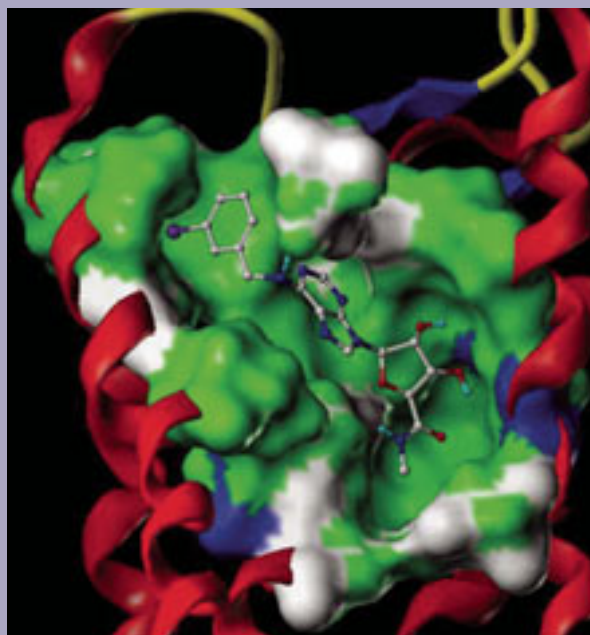
One way cells communicate with one another is through the secretion of small molecules including proteins, carbohydrates, lipids, or nucleosides such as adenosine. These molecules are synthesized and released by cells of almost all types. They may remain in the vicinity of the cell from which they are secreted and have an impact on neighboring cells, or they may travel through the blood stream and have effects on tissues far away.

In order for a cell to respond to a given signaling molecule, it must possess the appropriate receptor to detect it. Once the signaling molecule, or “ligand,” binds its

receptor, the receptor undergoes some kind of change. It may change shape, dissociate itself from some proteins and associate itself with new ones, modify other molecules, or perform a host of other possible actions that “tell” the cell that the ligand is present. This change initiates a cascade of events that results in a cellular response—cell division, specialization, or even death, depending on the ligands, receptors, and cell types involved. Ligand/receptor interactions can be fantastically complex. A given ligand may bind more than one kind of receptor. A given receptor may bind more than one kind of ligand. Different receptors may interact with one another. Different cell types may respond differently to the same ligand. The panoply of activity reflects the enormous complexity and sophistication of cellular communications and responses.

The structure of many receptors is fairly straightforward. In a basically linear chain of amino acids, one end of the receptor sticks outside the cell, a short central region spans the membrane, and the other end of the receptor is inside the cell. Receptors for adenosine, in contrast, are members of the seven transmembrane (7TM) receptor family, so-called because these receptors cross back and forth through the cell membrane a total of seven times. In humans, the 7TM receptor family is believed to contain at least 600 members that are found in a wide range of cells involved in processes as diverse as sight, smell, nerve signaling, and regulation of hormones.

Adenosine signaling plays an important role in a number of crucial physiologic processes, including the proper functioning of the cardiovascular system. In the heart, under normal conditions, the adenosine-containing ATP is broken down to provide energy to power the life-sustaining contractions of cardiac muscle. However, during periods of prolonged elevated heart rate, ATP may be metabolized into a free form, which can then exit the cells. Once outside the cells, adenosine binds to its receptor on the surface of the heart cells, and acts to naturally slow the contractions of the muscle. This feedback loop protects the cardiac muscle against damage that could arise from chronic overstimulation of the heart.



Dr. Ken Jacobson and the researchers of NIDDK's intramural Molecular Recognition Section are using molecules that can bind to and activate (or inhibit) human proteins called adenosine receptors to study receptor signaling. Shown is a computer-generated model illustrating how one such activating molecule (an “agonist”) binds to one subtype of adenosine receptor. This image, a closer view of the larger image shown at the beginning of this chapter, shows the details of the interaction of the agonist with the binding site of the receptor. Image courtesy of Dr. Ken Jacobson, Chief, Molecular Recognition Section, NIDDK Division of Intramural Research.

Adenosine also plays another, perhaps more crucial role in the heart. A heart attack, or myocardial infarction, occurs when one of the arteries supplying blood to the heart muscle is blocked. When cardiac muscle downstream of the blockage is deprived of fresh blood and the oxygen it carries, it is damaged and may die. If the blockage is transient and blood flow is quickly restored, the damage to the heart muscle may be minimized and the heart can continue to beat. However, if the blockage is prolonged and blood is cut off for an extended period of time, the damage may be so great that the organ can no longer function; the heart attack is fatal. Scientists have observed that multiple, brief periods of oxygen deprivation—such as those resulting from transient blockages—have the curious effect of

significantly reducing the damage to cardiac muscle cells caused by a subsequent, lengthier blockage in culture and animal models. Importantly, this protective effect, called “preconditioning,” is not seen in experimental models where the binding of adenosine to its receptor is blocked. These findings suggest an important role for adenosine signaling in protecting cardiac muscle from transient oxygen deprivation.

At first glance, it might seem prudent to study whether it is beneficial to give people at risk for a heart attack adenosine itself. The problem with this approach is that there are four related receptor subtypes for adenosine—denoted A_1 , A_2A , A_2B , and A_3 —and each subtype is capable of eliciting its own unique response. A significant challenge for researchers and clinicians is to identify the relationship between activation of a specific adenosine receptor subtype and a particular cellular response, because perpetually elevated levels of adenosine given to protect the heart could have deleterious effects on other tissues.

The drug theophylline, used widely to treat asthma, illustrates the kind of problems that can arise with non-specific activation of adenosine receptors. Theophylline has a structure similar to adenosine and relaxes the bronchial tubes in the lungs to ease breathing. It is thought to act through the A_2B adenosine receptor subtype. However, in the kidneys and brain, acting through the A_1 receptor, theophylline works as a diuretic and can cause sleep disruption. These side effects make it a less-than-ideal treatment. A better treatment for asthma would have theophylline’s beneficial effect in the lungs, but not its unwanted effects in the kidneys and brain. But how would one go about designing such a drug?

Research at the Interface of Chemistry and Medicine

The NIDDK’s Dr. Ken Jacobson studies the structure and function of adenosine and other nucleotide receptors and the ways in which signaling through these receptors might be modulated as therapy. Historically, the use of engineered adenosine receptor activators (agonists) and inhibitors (antagonists), has been constrained in research

studies and in the treatment of patients because of limited knowledge of the receptors’ structures. Their usefulness has also been limited by the lack of receptor subtype specificity for many potential agonists or antagonists. Dr. Jacobson and his team of scientists in the Molecular Recognition Section have worked to design modified ligands that activate specific adenosine receptor subtypes as part of an overarching investigation into the structure/function relationship that underlies the adenosine/receptor pair. Though primarily used as research tools today, such molecules could one day be used as novel therapies for diseases in which cell signaling through adenosine receptors is disrupted. Such novel therapies could be designed to have greater specificity and fewer side effects than current adenosine receptor-targeting drugs.

A significant impediment to studies of signaling through adenosine receptors is the lack of knowledge regarding their three-dimensional structure; in fact, the detailed structure of most 7TM receptors is largely unknown. The unusual, multiple membrane-spanning stretches of the protein make these molecules ill-suited for methods, such as X-ray crystallography, that have been traditionally used to probe structure/function relationships in biomolecules. In fact, there is only one 7TM receptor for which a crystal structure has been solved—the photoreceptor rhodopsin, which is found in the eye. However, advances in computer technology and computing power have allowed Dr. Jacobson and his team of researchers to use the structure of rhodopsin as a starting point to generate models of 7TM receptors such as those for adenosine. This iterative process involves computer modeling of the interactions between adenosine and its receptors. This information can then guide the chemical and molecular biological synthesis of ligands or receptors with the incorporation of subtle alterations. Experimentally measuring the impact of these alterations on the ligand-receptor interaction and using the data generated in these studies help to further refine the computer model. After multiple rounds, this process results in the emergence of molecules that have been rationally designed to

activate or inactivate a receptor upon binding. These are highly potent, highly specific synthetic agonists or antagonists for a given receptor subtype.

The researchers in the Molecular Recognition Section have used this sophisticated approach to begin to elucidate at the molecular level the nature of the interactions between ligands and receptors. They have designed and successfully tested selective, potent agonists or antagonists for all four subtypes of adenosine receptors. Many of these molecules are especially valuable research tools because they are effective in cells obtained from many species. Thus, researchers working with cells derived from mouse, rat, rabbit, cow, or humans can all use these molecules in their studies.

The development of subtype-specific adenosine receptor agonists and antagonists has led Dr. Jacobson's team into a number of fruitful collaborations with scientists external to the NIDDK. Working with researchers at the University of Connecticut Health Center, Dr. Jacobson's group explored the molecular basis of the cardioprotective effective of adenosine described earlier. Using cultured chick cells, the scientists found that specific agonists of the A₁ receptor subtype offered short-term protection against oxygen deprivation, while agonists specific for the adenosine A₃ receptor subtype offered long-term protection. However, when both A₁ and A₃ agonists were present, an additional protective effect was seen. This finding could lead to the development of adenosine receptor agonists specifically tailored to have a protective effect in people at risk of a heart attack and simultaneously avoid the problems associated with less-specific agonists.

Future of Adenosine and Adenosine Receptor Engineering

Dr. Jacobson's future research goals are far more ambitious than devising new ways to activate nucleoside receptor subtypes. Dr. Jacobson, his research team, and his collaborators are hard at work exploring the possibility of synthesizing unique, customized ligand-receptor pairs to facilitate targeted cell signaling.

Properly designed, each customized ligand would interact only with its customized receptor, and *vice versa*. These molecules, called "neoreceptors" by Dr. Jacobson, would largely avoid the problems associated both with receptors that bind multiple ligands and with ligands that bind multiple receptor subtypes. Such an approach could also minimize the likelihood of unwanted side effects from drug treatment, because the only tissue or organ that would be capable of responding to the customized ligand would be the one(s) bearing the customized neoreceptor. This pioneering work, still in its earliest stages, will need further refinement before it is ready to move out of the laboratory and into the clinical setting. However, it has shown promise in some preliminary studies in cultured cells. Using this approach, Dr. Jacobson and his team have provided a cardioprotective effect in heart cells similar to that seen with selective adenosine receptor agonists. Translation of these laboratory discoveries into clinical research applications is an ultimate goal of this research.

Currently, there is no reliable way to deliver these neoreceptors in a targeted fashion in humans, so therapies based on neoreceptors are years away. To be successful, this approach will require advances in targeted gene therapy in order to deliver the neoreceptors to their target tissue, so that they can signal the presence of their customized ligand. Nevertheless, this novel, highly exploratory research by the NIDDK sets the stage for rational drug development by providing the tools and raw materials for future studies. It represents an excellent example of how the most fundamental studies can plant the seeds for future treatment strategies. As a whole, Dr. Jacobson's research illustrates how something as ordinary and widely-consumed as caffeine can lead to insights into molecular signaling, how research into the pathways influenced by caffeine can open the door to treatments for a number of devastating and costly diseases, and how creative minds can use these insights to fashion new therapies that may one day permit the ultimate in customization of drugs and their targets.

Think about that over your next cup of coffee.